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SHORT COMMUNICATION

Reduction of Myocardial Infarct Size by Dronedarone in Pigs—A Pleiotropic Action?

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Abstract
Purpose Dronedarone is a first-line drug to prevent the recurrence of atrial fibrillation according to ESC guidelines. In the recent ATHENA trial, dronedarone reduced mortality and also hospitalization for acute coronary syndrome in patients with atrial fibrillation. This beneficial effect suggests that dronedarone might have also an impact on events associated with ischemia/reperfusion injury.
Methods Fourteen anesthetized pigs received either dronedarone (2.5 mg/kg) or placebo. Effects of dronedarone on heart rate and blood pressure were reversed by atrial pacing and aortic constriction before pigs were subjected to 90 min regional low-flow myocardial ischemia and 2 h reperfusion. Regional myocardial blood flow was measured with microspheres and infarct size determined by TTC staining.
Results With comparable heart rate and left ventricular pressure during ischemia, dronedarone reduced infarct size from 34±3% to 22±4% ($p<0.05$) of the area at risk. Subendocardial blood flow during ischemia was not different between groups. The relationship between ischemic subendocardial blood flow in the area at risk and infarct size was displaced downwards, reflecting a direct cardioprotective action of dronedarone.
Conclusion The beneficial effect of dronedarone is attributed to cardioprotective properties, possibly through attenuation of calcium overload during myocardial ischemia/reperfusion.

Key words Dronedarone · Infarct size · Myocardial ischemia · Reperfusion

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Introduction

Atrial fibrillation is the most frequent type of cardiac arrhythmia, with an increasing prevalence in elderly patients. Atrial fibrillation is associated with increased mortality, risk of stroke, and impaired quality of life. According to the recent ESC guidelines for the management of atrial fibrillation, dronedrone is a first-line drug to prevent the recurrence of atrial fibrillation [1].

The electrophysiological properties of dronedarone are similar to those of amiodarone, but dronedarone is free of iodine and less toxic. Dronedarone acts on several ion channels in the cardiomyocyte sarcolemma, and it is used to inhibit the repolarizing potassium current [2]. In contrast to amiodarone, dronedarone exerts no effect on the sodium-potassium-ATPase, but inhibits the sodium-calcium-exchanger [3].

In the ATHENA trial, dronedarone reduced the incidence of cardiovascular death from 3.9% to 2.7% in patients with atrial fibrillation and additional risk factors [4]. This beneficial outcome in terms of mortality, notably also in hospitalization for acute coronary syndromes, distinguishes dronedarone from other anti-arrhythmic agents and suggests a potential impact on events associated with ischemia/reperfusion injury.

An interaction between atrial fibrillation and ischemic heart disease is established. Myocardial ischemia per se increases excitability and decreases conduction velocity. The ischemia-induced loss of contractile function and myocardial stretch add to increased excitability and slowed conduction, and the loss of viable atrial tissue with replacement by fibrosis further decreases conduction velocity. Vice versa, atrial fibrillation promotes myocardial ischemia. Apart from tachycardia which increases myocardial oxygen consumption and decreases diastolic duration

[5], atrial fibrillation increases cardiac catecholamine release and induces α-adrenergic coronary vasoconstriction [6]. In the presence of a coronary stenosis, the α-adrenergic coronary vasoconstriction secondary to atrial fibrillation further decreases coronary blood flow and augments myocardial ischemia [7, 8]. Part of the beneficial effect of dronedarone might therefore be related to its ability to attenuate α-adrenoceptor responses to catecholamines [9].

It is entirely unclear at present whether or not dronedarone, apart from its potential effects on coronary blood flow, has direct cardioprotective properties in the setting of myocardial ischemia/reperfusion. A potential infarct size reduction by dronedarone might thus be secondary to a) heart rate reduction [10], b) coronary vasodilation [11] and/or c) an intrinsic cardioprotective property of dronedarone.

We have therefore now tested the impact of dronedarone on myocardial infarct size as the endpoint of protection in a clinically relevant model of controlled myocardial ischemia and reperfusion in anesthetized pigs [12], which permits control of heart rate, blood pressure, and coronary blood flow.

Methods

The experimental protocols were approved by the Bioethical Committee of the district of Düsseldorf (G 1136/10).

Experimental preparation

The experimental preparation has been described in detail recently [12]. In brief, fourteen Göttinger minipigs (20–40 kg) were sedated with ketamine and anesthetized with thiopental (500 mg i.v.). Anesthesia was then maintained using enflurane (1–1.5%) with an oxygen/nitrous oxide mixture (40%:60%). The common carotid arteries were cannulated to measure arterial pressure and to supply blood to an extracorporeal perfusion circuit. The jugular veins were cannulated for volume replacement. After a left lateral thoracotomy the heart was exposed and instrumented with a micromanometer (P7, Konigsberg Instr., Pasadena, CA, USA) placed in the left ventricle. A fabric band was placed around the descending thoracic aorta to control left ventricular peak pressure by aortic constriction. Pigs were anticoagulated with 20,000 IU sodium heparin and additional doses of 10,000 IU at 2 h intervals. The left anterior descending (LAD) coronary artery was cannulated and perfused from the extracorporeal perfusion circuit. Coronary arterial pressure was measured from the sidearm of the coronary cannula and held above 75 mmHg to avoid hypoperfusion prior to ischemia by adjusting the roller pump. Heart rate was controlled by left atrial pacing (Hugo Sachs Elektronik Type 215/T, Hugstetten, Germany).

Regional myocardial blood flow

Radiolabeled microspheres (15 μm in diameter; ^{46}Sc; Perkin Elmer, Waltham, MA, USA) were injected into the coronary perfusion circuit to determine the area at risk and regional myocardial blood flow (Wizzard 2740, Perkin Elmer, Waltham, MA, USA). Subendocardial blood flow to the inner myocardial layers, where most of the infarction and a potential redistribution of flow occur, is reported.

Infarct size

At the end of each experiment, the heart was sectioned from base to apex into 5 transverse slices in a plane parallel to the atrioventricular groove. Slices were immersed in 0.09 mol/L sodium phosphate buffer containing 1.0% triphenyl tetrazolium chloride (Sigma-Aldrich Chemie Gmbh, Munich, Germany) and 8% dextran for 20 min at 37°C. The amount of infarcted tissue is expressed as percent of the area at risk, as defined by microspheres.

Drug administration

Dronedarone (2.5 mg/kg body weight) was dissolved in 15 ml 1:2 saline/PEG400 (Sigma Aldrich, Schnelldorf, Germany). In preliminary experiments, the i.v. infusion of dronedarone caused generalized vasodilation, as characterized by hypotension and increased coronary blood flow. To minimize such initial vasodilator effect of dronedarone, the infusion was then fractionated. During infusion (1 ml/min), coronary perfusion pressure was held above 75 mmHg. After injection of 5 ml dronedarone solution, the infusion was stopped. As soon as coronary blood flow had returned to baseline and systemic hemodynamics had reached a steady state, the infusion was continued. The heart rate reduction by ten beats/minute with our acute i.v. administration of dronedarone was somewhat more pronounced than that of four beats/minute with chronic oral administration in the ATHENA trial [13], but in a similar range. To control for effects of the solvent per se, 15 ml of 1:2 saline/PEG400 were given i.v. prior to the start of the protocol in each experiment; this placebo solution caused no measurable effect.

Experimental protocol

Dronedarone (n= 6) After administration of dronedarone, heart rate and left ventricular pressure were restored back to baseline values by atrial pacing and aortic constriction, respectively. Coronary inflow was then reduced to 10% of baseline and maintained constant. At 5–10 min ischemia, a

Table 1 Systemic hemodynamics

	Time	HR [1/min]	LVPmax [mmHg]	dPdtmax [mmHg/s]	CAPmean [mmHg]	CBFmean [ml/min]
Dronedarone (=6)	Baseline	88±8	101±2	1,648±164	132±11	18.9±1.4
	PEG	88±8	101±3	1,599±141	135±7	19.9±1.5
	Dronedarone	78±4#	80±4#*	814±54#*	110±5	21.9±1.5
	Match	91±7	101±2	858±42#*	132±5	19.4±1.4
	Isch5	90±7	81±4*	731±33*	26±3*	2.0±0.1*
	Isch85	96±6	92±4	986±57*	34±7*	2.0±0.1*
	Rep10	99±6	80±5*	923±103#*	116±8*	47.0±8.0*
	Rep30	104±7*	78±4*	1,007±56#*	113±7*	35.0±4.9*
	Rep60	106±5*	79±3*	1,063±29#*	114±6*	31.0±4.2#*
	Rep120	110±7*	76±2*	1,155±100*	120±8	27.9±3.4#*
Placebo (n=8)	Baseline	92±3	93±3	1,411±56	121±3	20.8±1.5
	PEG	93±1	94±3	1,388±61	124±3	21.5±2.2
	Placebo	93±1	92±2	1,376±65	116±4	20.6±2.0
	Match	91±2	93±2	1,346±47	118±8	20.8±1.8
	Isch5	93±1	77±3*	982±54*	23±2*	2.3±0.3*
	Isch85	102±5	83±4*	1,165±88*	34±8*	2.3±0.3*
	Rep10	105±7*	80±1*	1,325±118	113±6	40.4±3.5*
	Rep30	103±5	80±4*	1,343±178	119±9	39.9±3.6*
	Rep60	113±4*	78±3*	1,448±120	108±4	40.7±3.3*
	Rep120	113±5*	75±3*	1,309±111	108±6	39.1±3.9*

PEG: after i.v. injection of PEG400; Dronedarone/Placebo: steady state after complete dronedarone/placebo infusion; Match: restoration of baseline values by atrial pacing and aortic constriction; Isch5/85: 5/85 min after the onset of ischemia; Rep10/30/60/120: 10/30/60/120 min reperfusion

HR heart rate; *LVPmax* maximal left ventricular pressure; *dPdtmax* maximal rate of rise of left ventricular pressure; *CAPmean* mean coronary arterial pressure; *CBFmean* mean coronary blood flow

*$p<0.05$ vs. Baseline; #$p<0.05$ vs. Placebo (two-way ANOVA and Fisher's LSD post-hoc tests)

measurement of regional myocardial blood flow was performed. After 90 min ischemia the myocardium was reperfused for 2 h.

Placebo (n= 8) The protocol was identical to that of the dronedarone group, except that the infusion of dronedarone was replaced by saline.

Statistics

Data are mean±SEM. Hemodynamics were analyzed by two-way ANOVA for repeated measures. When a significant difference was detected, individual mean values were compared by Fisher's post-hoc tests (LSD). Area at risk, infarct size, and subendocardial blood flow during ischemia were compared by Student's *t*-tests. Linear regression analyses between ischemic subendocardial blood flow in the area at risk and infarct size were performed separately for dronedarone and placebo and then compared by ANCOVA. Differences were considered significant at the level of $p<0.05$.

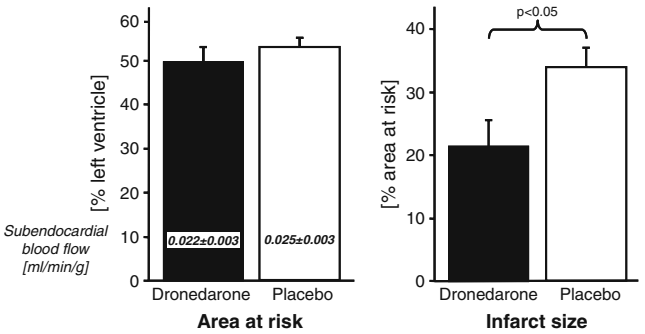

Fig. 1 Area at risk, infarct size, and subendocardial blood flow during ischemia with dronedarone and placebo

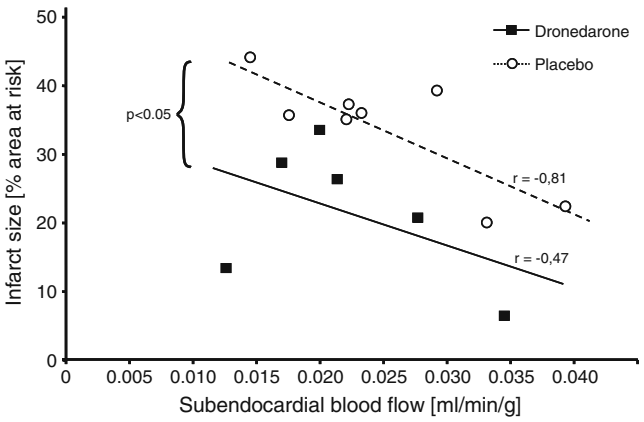

Fig. 2 Infarct size as a function of subendocardial blood flow during ischemia. With dronedarone, the relationship is shifted downwards (ANCOVA), reflecting reduced infarct size for any given residual blood flow

Results

Ventricular extrasystoles were observed during ischemia/reperfusion in most pigs, but not different between groups. Three pigs experienced ventricular fibrillation, two pigs with dronedarone during the first minutes of reperfusion and one pig with placebo after the onset of ischemia; in all cases, ventricular fibrillation was immediately terminated by DC countershock.

Hemodynamics at baseline were not different between dronedarone and placebo (Table 1), and the infusion of saline/PEG400 solvent did not alter systemic hemodynamics. Dronedarone decreased heart rate, left ventricular pressure, and left ventricular dP/dt. With atrial pacing and aortic constriction, heart rate and left ventricular pressure were restored back to baseline and not different from those with placebo for the remaining protocol. Left ventricular dP/dt remained lower but was not different from placebo during ischemia. The area at risk, expressed as percent of the left ventricle, and subendocardial blood flow during ischemia were not different between dronedarone and placebo. Infarct size was 22%±4% of the area at risk with dronedarone and 34%±3% ($p<0.05$) with placebo (Fig. 1). The relationship between subendocardial blood flow and infarct size was significantly displaced downwards with dronedarone (Fig. 2), reflecting a direct cardioprotective action of dronedarone independent of its potential effect on residual blood flow.

Discussion

In the present study dronedarone reduced myocardial infarct size (Fig. 1). In our constant flow preparation, dronedarone did not improve blood flow or its distribution. The observed infarct size reduction was independent from residual blood flow during ischemia, as reflected by the downwards displacement of the flow/infarct size relationship with dronedarone (Fig. 2). While admittedly we cannot exclude that atrial pacing or aortic constriction per se have an effect on infarct size, we used atrial pacing and aortic constriction to match heart rate and blood pressure in the dronedarone group to those in the placebo group and can therefore exclude that the protection by dronedarone was secondary to heart rate or blood pressure reduction.

While the present study clearly demonstrates a direct cardioprotective action of dronedarone in a highly relevant model of regional myocardial ischemia/reperfusion in pigs, a species with a similar spatial and temporal development of infarction as humans, and a high translational value [12, 14], it does not provide a mechanistic explanation for such cardioprotection. Among the several ion channels which are inhibited by dronedarone, the inhibition of the sodium-calcium exchanger [3] might be of particular importance as this would reduce calcium overload during myocardial ischemia/reperfusion and thus contribute to the maintenance of viability [15]. Pharmacological inhibition of sodium-calcium exchange has indeed been shown to decrease calcium overload and hypercontracture in rat cardiomyocytes and to reduce infarct size in pigs [16]. However, we currently have no proof for this potential explanation, and thus the cardioprotective effect of dronedarone remains "pleiotropic" at this point. We have previously reported reduced infarct size through a heart rate-independent, direct cardioprotective, "pleiotropic" action of ivabradine, an agent which also acts on ion channels not only in the sinus node [5, 17, 18]. In fact, dronedarone also inhibits I_f channels at a similar potency as ivabradine [19]. Since, however, we do not know the mechanism underlying protection by either ivabradine or dronedarone, we also do not know whether these drugs have a common mechanism to induce cardioprotection. More reductionist models may be better suited to identify the mechanistic background for our observation of direct cardioprotection [15]. Nevertheless, our present results on dronedarone support the observation from the ATHENA trial, that mortality from cardiovascular events is reduced with dronedarone [4].

Conflict of interest The present study was supported by an unrestricted educational grant from sanofi-aventis Germany GmbH. GH has received honoraria for lectures from sanofi-aventis Germany GmbH.

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